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ORIGINAL PAPER

Synthesis of thiazolo[3,2-*a*]pyrimidine molecules, in vitro cytotoxic evaluation and molecular docking studies

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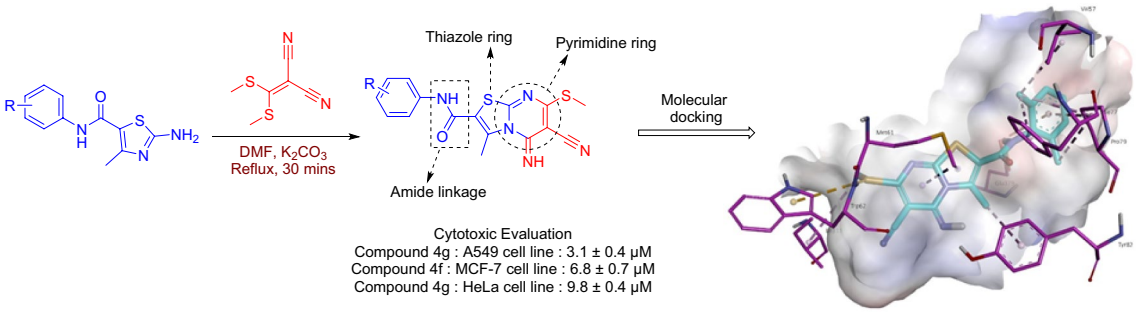
Abstract

Novel hybrid molecules of thiazolopyrimidine **4a–j** have been prepared starting from various thiazoles **3a–j**. The reaction of thiazoles **3a–j** with thiourea yielded hybrid molecules **4a–j** in an excellent yield. These molecules were screened for their anticancer activities against human breast carcinoma cell line (MCF-7), human lung adenocarcinoma cell line (A549) and human cervical cancer cell line (HeLa) using MTT assay. Among all molecules, compounds **4g** and **4f** exhibited potent cytotoxic activity. Compound **4g** with IC_{50} value of 3.1 ± 0.4 µM and IC_{50} value of 9.8 ± 0.4 µM against A549 and HeLa cell line, respectively. Compound **4f** with IC_{50} value of 6.8 ± 0.7 µM against MCF-7 molecular docking study of all synthesized molecules **4a–j** was performed on topoisomerase II using the AutoDock technique. All the synthesized thiazolopyrimidine hybrid molecules have been characterized and confirmed using spectroscopic techniques.

Graphical abstract



Keywords Thiazolopyrimidine · Hybrid molecules · Topoisomerase II · Anticancer activity · Molecular docking

Introduction

Cancer is the most threatening ailment in that abnormal cells divide rapidly, and it can result in tumors, harm the immune system and damage the body complex [1]. So many persons die every year from cancer [2]. Existing movements for cancer management were inadequate because of reasonably numerous serious side outcomes [3]. In spite of the accessibility of several anticancer medicines, growth in emerging novel anticancer agents for handling cancer without side reactions is an important aim for researchers [4]. Heterocyclic molecules are attracting the vast attention of researchers over the years because of their great therapeutic uses [5]. Heterocyclic molecules are ideal for preparing molecules, which will react with the target sites of cancer cells and disrupt their genetic path link [6].

Thiazolopyrimidine is a hybrid molecule containing of pyrimidine and thiazole heterocyclic rings and is a fortunate moiety found in several natural products and many synthetic molecules. There are several bioactive thiazolopyrimidines that possess anticancer activity (**I–V**, Fig. 1) [7]. Many types of cancer are presently treated using the anticancer

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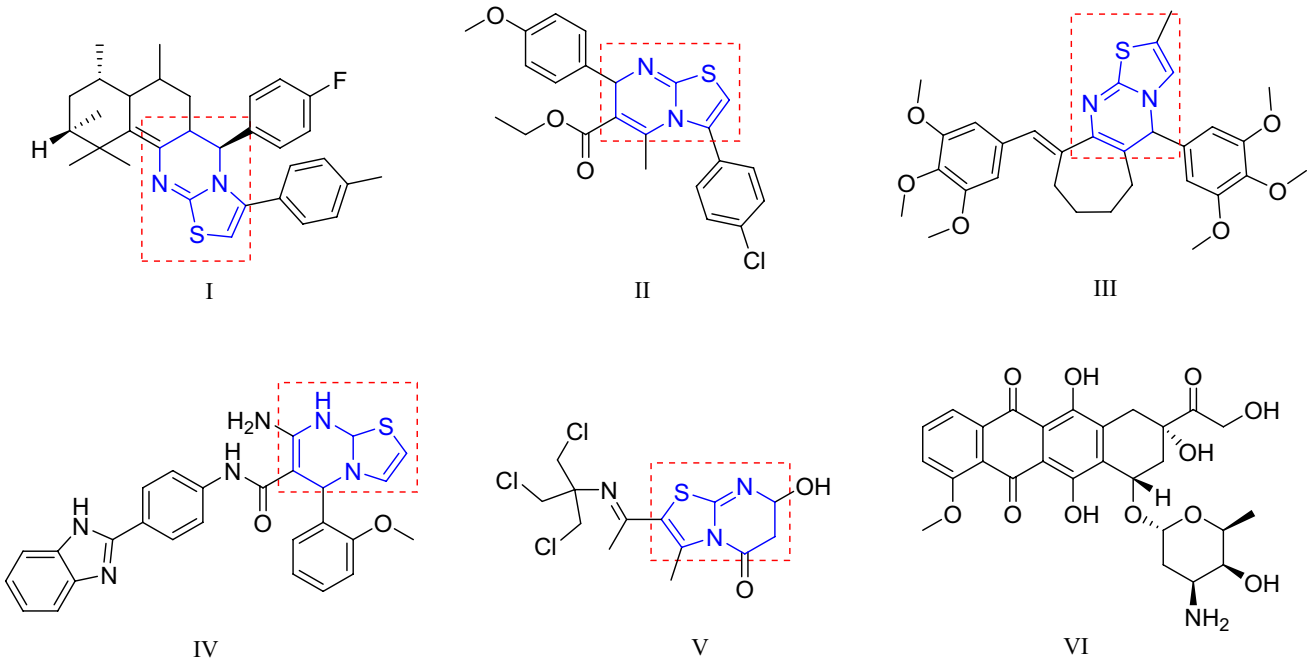


Fig. 1 Bioactive fused thiazolopyrimidines (**I–V**) and structure of doxorubicin (**VI**)

drug doxorubicin (**VI**, Fig. 1). However, its usage has been restricted by elements including medication resistance, toxicity and congestive heart failure that have been documented in certain individuals [8]. Moreover, thiazolo[3,2-*a*]pyrimidine has appeared as a potential synthetic area in heterocyclic synthesis [7, 9–14] due to its omnipresent applications as acetylcholinesterase inhibitor [15], calcium antagonist [16], cytotoxic agent [17], antihypertensives [18], antiproliferative [19], Mycobacterium tuberculosis O-acetylserine sulfhydrylase inhibitor [20], anticancer [21, 22], NK-2 antagonist [23], antitubercular[24], antitumor [25], antiviral[26], anti-inflammatory and antinociceptive [27] as well as in polymerization reactions as copolymer [28, 29].

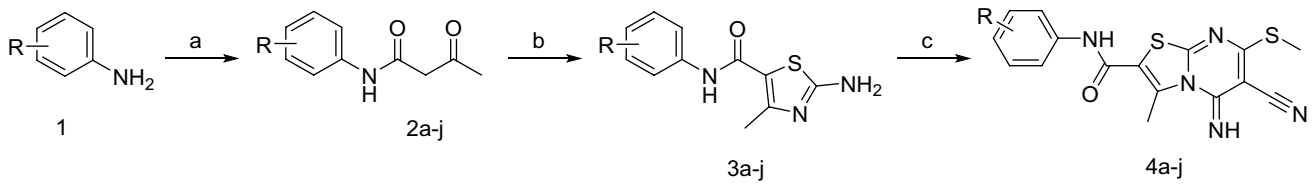
Over the past two decades, there has been a wide interest increased in the synthesis of hybrid molecules [30–32] and their assessment as variety of strong therapeutical and pharmacological agents. Hybrid molecules are stable molecular fusion of two biologically important molecules operating at separate sites and are a potential approach to treating complicated multifactorial disorders [33–35]. Two different molecular components are combined to form these "dual-acting compounds." Motivated by aforementioned activities of thiazolopyrimidines, we planned to synthesize some hybrid molecules comprising these two units. The newly developed molecules may be employed in very effective anticancer drugs since the hybridization of these two different biologically active molecules may have a synergic impact. Our continuous research in the field of synthesis of various bioactive heterocyclic compounds [36–38] encouraged us to develop some novel hybrid molecules of thiazolo[3,2-*a*] pyrimidine for medicinal interest.

Results and discussion

Chemistry

To find novel anticancer molecule and synthesis of different heterocyclic molecules, here, we report ten newly synthesized molecules with thiazolo[3,2-*a*]pyrimidine in their main structure. The compounds **4a–j** were elucidated through inspecting their spectroscopic data like ^{1}H-NMR,^{13}C-NMR, FTIR and mass spectroscopy. In the first step, 3-oxo-*N*-arylbutanamide and *N*-bromosuccinimide reacted at ambient temperature to get 2-bromo-3-oxo-*N*-arylbutanamide. Then, thiourea was added for ring closure to obtain 2-amino-*N*-aryl-4-methylthiazole-5-carboxamide **3a–j**.

Then, compound **3a–j** was reacted with bis(methylthio) methylene malononitrile to obtain novel and highly functionalized thiazolo[3,2-*a*]pyrimidine **4a–j** derivatives as appear in Scheme 1. The ^{1}H-NMR graph of compounds presented that 4-methyl thiazole protons were detected at s 2.20–2.57 ppm (CH_3) as a singlet, at s 2.94–3.05 ppm (SCH_3) for pyrimidine thiomethyl protons, which were singlet peaks. The aromatic region was seen between 6.94 and 7.83 ppm. A smaller singlet peak seen at s 9.03–10.64 ppm (NH) indicated the pyrimidine imine proton. Acetamide protons were observed at s 9.81–10.80 ppm (NH) as a singlet.



Scheme 1 Reagents and conditions: **a** ethyl acetoacetate, KOH, reflux, 24h, **b** NBS, thiourea, MeOH, reflux, 4h, **c** bis(methylthio)methylene malononitrile, K_2CO_3, DMF, reflux, 30 min

To optimize the reaction conditions for the synthesis of compound **4a–j**, various bases, such as anhydrous potassium carbonate and triethylamine, were utilized in respective solvents, such as methanol, ethanol, tetrahydrofuran and acetonitrile. As a result, we found that the reaction of **3a–j** with bis(methylthio)methylene malononitrile was faster and afforded the thiazolo[3,2-*a*]pyrimidine **4a–j** in good yield in the presence of potassium carbonate and DMF. Furthermore, the reaction of thiazolo[3,2-*a*]pyrimidine with different primary and secondary amines or phenols was not promising to yield substituted thiazolo[3,2-*a*]pyrimidine derivatives because of the poor reactivity of SMe, so maybe oxidation of the sulfides to sulfones can transfer it into good leaving group, thus allowing good reactivity, and further derivatives of thiazolo[3,2-*a*]pyrimidine can be produced. It has also been observed that the reaction of thiazolo[3,2-*a*]pyrimidine with hydrazine hydrate and phenyl hydrazine yielded a mixture of cyclized and noncyclized products. Due to high nitrogen content, lipophobicity and poor solubility in a variety of solvents, the purification of the product was not possible. The one-pot reaction of acetoacetanilide, *N*-bromosuccinimide and thiourea followed by the addition of bis(methylthio) methylene malononitrile was not clean and did not yield the desired product (Fig. 2).

Initially, the experiment was done without using any solvent or catalyst at 90 °C, but the outcome was none (Table 1, entry 1). Therefore, next reaction was executed using water as a solvent and without a base at ambient temperature for 1h, but product formation does not occur (entry 2). So, with the addition of potassium carbonate, mass was refluxed for 1h but the result was again disappointing, no product formation was observed (entry 3). Then, triethylamine was used as a base and acetone as solvent refluxed for 1h, leading to 12% of the product (entry 4) and 10% product formation when potassium carbonate was used as a base (entry 5).

Reaction state was then changed regarding solvent using methanol and triethylamine as a base. This resulted in the product being obtained in 10% yield (entry 6), and as a base, when potassium carbonate was used, the yield was 12% (entry 7). Further optimization of the reaction was done by using ethanol as a solvent and triethylamine as a base. Then, the mass was refluxed for 1h and product was obtained in 38% yield (entry 8) and 43% yield was obtained when potassium carbonate was used as a base (entry 9). Subsequently, the use of tetrahydrofuran as a solvent and triethylamine as a base produced a product of 32% (entry 10), while the use of potassium carbonate produced a yield of 38% (entry 11). Surprisingly, when using acetonitrile as a solvent and triethylamine as a base, the yield was 45% (entry 12), and when potassium carbonate was used as a base, 59% yield (entry 13) was archived. When N, N-dimethylformamide was used

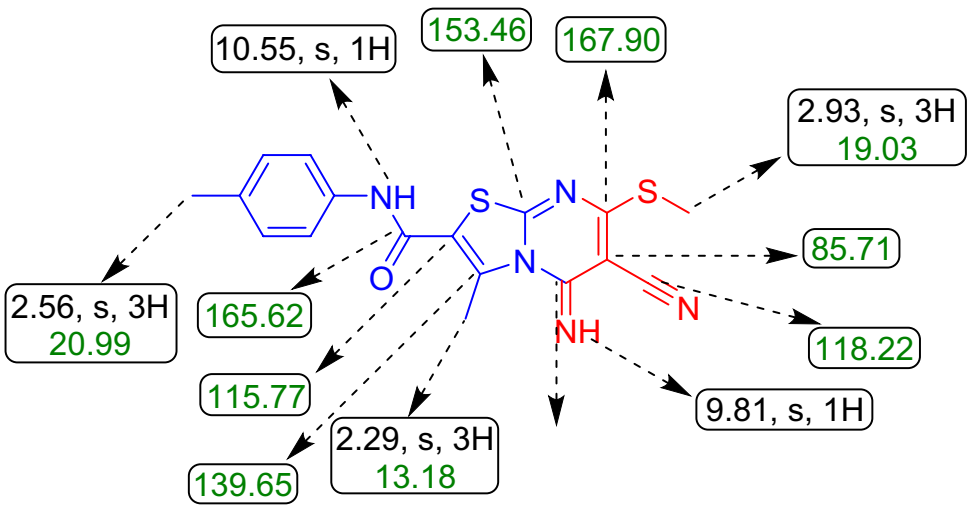


Fig. 2 Selected ^{1}H (black) and ^{13}C NMR (green) of compound 4b

Table 1 Optimization of the reaction conditions

Entry	Solvent	Base[a]	Temp. (°C)[b]	Yield (%)[c]	Purification necessary/by-product formation
1	No solvent	–	90	–	–
2	H_2O	–	rt	–	–
3	H_2O	K_2CO_3	Reflux	–	–
4	Acetone	Et_3N	Reflux	12	Yes
5	Acetone	K_2CO_3	Reflux	10	Yes
6	MeOH	Et_3N	Reflux	10	Yes
7	MeOH	K_2CO_3	Reflux	12	Yes
8	EtOH	Et_3N	Reflux	38	Yes
9	EtOH	K_2CO_3	Reflux	43	Yes
10	THF	Et_3N	Reflux	32	Yes
11	THF	K_2CO_3	Reflux	38	Yes
12	MeCN	Et_3N	Reflux	45	Yes
13	MeCN	K_2CO_3	Reflux	59	Yes
14	DMF	Et_3N	Reflux	62	Yes
15	DMF	K_2CO_3	Reflux	95	No

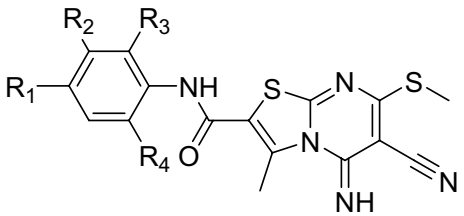
[a]Amount of base was 1 equivalent

[b]Reaction time was 1h

[c]Yield is given for isolated product without purification

with triethylamine, this yielded a 62% yield (entry 14), but when potassium carbonate as a base reaction mixture was heated to reflux for 30 min, the resultant yield was 95% (entry 15). This variation did not lead to the formation of by-products and gave an excellent yield with high purity. It was clearly observed that, while triethylamine overall yield was low compared to potassium carbonate. Solvents such as methanol and acetone reduced the yield of product, respectively (entries 2 and 3), using water as a green solvent, the experiment did not continue smoothly, maybe because of the poor solubility of the reactants in water. With the optimized reaction conditions, the technique was used to produce novel hybrid thiazolo[3,2-*a*]pyrimidine **4a–j** molecules (Table 2).

Table 2 Physicochemical characteristics of the thiazolo[3,2-*a*]pyrimidine molecules **4a–j**



Compound	R^1	R^2	R^3	R^4	Yield (%)	Melting point (°C)
4a	OCH_3	H	H	H	83	213–215
4b	CH_3	H	H	H	91	226–228
4c	H	H	H	H	72	201–203
4d	H	Cl	H	H	67	216–218
4e	CH_3	H	CH_3	H	87	242–244
4f	Cl	H	H	H	89	230–232
4g	H	H	CH_3	CH_3	65	238–240
4h	F	H	H	H	61	237–239
4i	Br	H	H	H	86	248–250
4j	H	H	OCH_3	H	90	212–214

Synthesized molecules **4a–j** were further investigated for molecular docking to determine the binding affinity of the molecules.

Cytotoxic assessment

All the newly synthesized hybrid thiazolopyrimidines **4a–j** were tested for their anticancer activity against human breast carcinoma cell line (MCF-7), human lung adenocarcinoma cell line (A549) and human cervical cancer cell line (HeLa) by MTT assay. The anticancer data are depicted in Table 3. It has been observed that nine molecules possess moderate-to-good anticancer activity against different cancer units. Compound **4a** having methoxy substitution on *para* position showed moderate activity against all cell lines. Compound **4b** having methyl substitution on *para* position showed increased cytotoxic activity in A549 cell line, but in MCF-7 and HeLa, the results were moderate. The phenyl ring attachment in compound **4c** showed least inhibition in HeLa. Compound **4d** having chloro substitution on *meta* position showed good activity against MCF-7, but showed least activity compared to all tested molecules in A549 cell line. Compounds **4e** and **4g** having methyl substitutions on the *ortho*, *para* and *meta*, respectively, exhibited excellent activity in all cell lines. In particular, molecule **4g** exhibited outstanding activity on A549 (3.1 ± 0.4), and compound **4f** having chloro substitution on *para* position displayed good activity on MCF-7 (6.8 ± 0.7). Compound **4g** also showed (9.8 ± 0.4) good results against HeLa cell line. Compounds **4h** and **4i** having fluoro and bromo substitution on *para* position showed moderate-to-good activity. Compound **4j** having methoxy substitution on *ortho* position also showed moderate cytotoxic activity. This result indicates that molecules with methoxy and halogen substitute on thiazolopyrimidine exhibited great anticancer activity. Therefore, these outcomes could be noteworthy in the development of potent and harmless anticancer molecules.

The IC_{50} value was calculated from the dose–response curve. The values (μM) denote the average ± SD of three different assessments. Doxorubicin was utilized as a positive control (Fig. 3).

Molecular Docking

Considering the excellent in vitro outcomes and to decide the possible binding sites of potent compounds, molecular docking was performed on topoisomerase II alpha using AutoDock Vina to study the affinity of the synthesized thiazolopyrimidines. The crystal structure of topoisomerase II alpha was downloaded from the Protein Data Bank with PDB id 1ZXM. The docking results of synthesized molecule displayed hydrogen bond with several amino acids. The main bonding amino acid was Arg241, Lys321, Lys168, Tyr73, Tyr82, Arg242, Glu379, Gln59 and Arg 324. The prepared 3D molecule structures were energy-minimized and utilized for docking studies. All newly prepared molecules were subjected to the docking. Table 4 shows the docking score with binding energy and active site residues with doxorubicin as standard. All prepared molecules exhibited decent binding energy with the target varying from − 7.9 to − 8.7 kJ mol^{-1}. Between all of the molecules, molecule **4e** had best docking value of − 8.7 to the standard doxorubicin. Among the experimented molecules, nitrile group of compound **4a** formed H-bonding with Arg B:241 and Lys B:321, and nitrile group also shows H-bonding with Lys A:168 in compound **4b**. In compound **4c,** two hydrogen bonds were formed with Tyr B:73 and Arg B:241. Compound **4d** having halogen atom did not form any H-bond, but formed a conventional and *van der Waals* bond with Trp B: 62 and Lys B:83. In thiazolopyrimidine **4e**, imine hydrogen formed the

Table 3 IC_{50} of the synthesized molecules on cancer cell lines

Compound	IC_{50} in μM					
	A549		MCF-7		HeLa	
	24h	48h	24h	48h	24h	48h
4a	10.2 ± 0.4	**8.4 ± 0.3**	17.4 ± 0.4	15.3 ± 0.2	15.3 ± 0.5	12.5 ± 0.5
4b	**7.4 ± 0.3**	**6.3 ± 0. 2**	15.7 ± 0.3	12.3 ± 0.2	17.3 ± 0.5	14.5 ± 0.3
4c	12.3 ± 0.3	10.5 ± 0.6	12.1 ± 0.3	10.1 ± 0.5	22.2 ± 0.3	20.1 ± 0.3
4d	15.5 ± 0.2	12.4 ± 0.4	9.3 ± 0.5	**7.3 ± 0.6**	19.7 ± 0.2	15.4 ± 0.2
4e	**6.4 ± 0.2**	**3.2 ± 0.5**	18.3 ± 0.4	14.2 ± 0.3	14.1 ± 0.4	10.3 ± 0.5
4f	13.5 ± 0.4	10.3 ± 0.3	9.5 ± 0.4	**6.8 ± 0.7**	15.8 ± 0.3	11.2 ± 0.7
4g	**6.1 ± 0.3**	**3.1 ± 0.4**	20.2 ± 0.2	17.5 ± 0.4	13.2 ± 0.6	**9.8 ± 0.4**
4h	10.1 ± 0.6	9.1 ± 0.3	9.3 ± 0.3	**7.1 ± 0.5**	14.6 ± 0.5	10.9 ± 0.1
4i	12.7 ± 0.4	10.2 ± 0.5	10.8 ± 0.4	**8.3 ± 0.3**	16.8 ± 0.2	14.4 ± 0.4
4j	11.5 ± 0.2	**8.5 ± 0.3**	17.9 ± 0.2	14.4 ± 0.6	16.2 ± 0.3	14.1 ± 0.3
Doxorubicin	0.9 ± 0.03	0.7 ± 0.04	1.4 ± 0.2	0.9 ± 0.02	0.6 ± 0.05	0.4 ± 0.03

The compounds which shows more potent activity are highlighted in bold

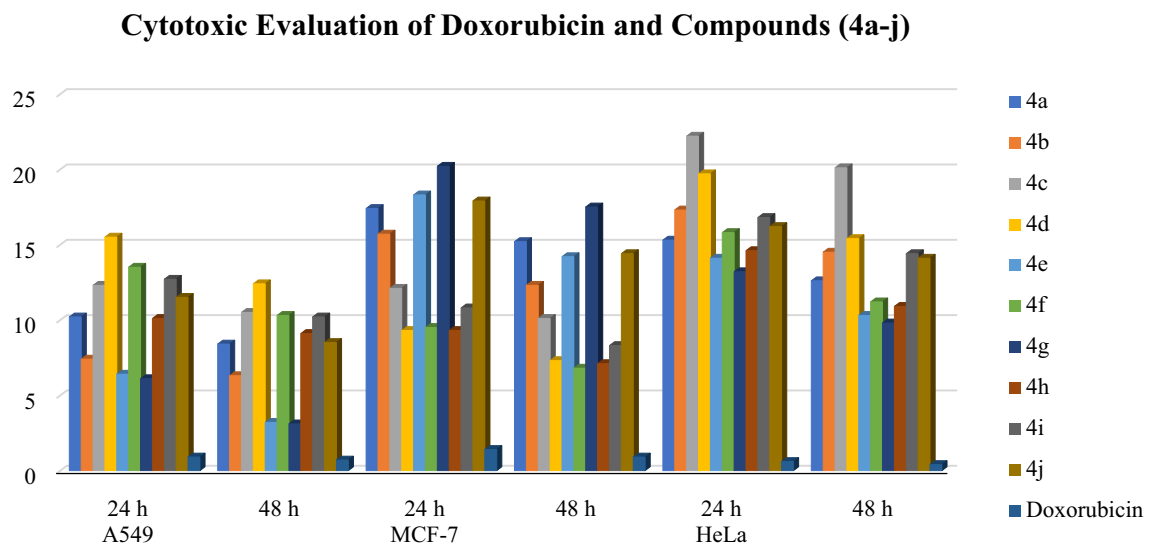


Fig. 3 Cytotoxicity evaluation of doxorubicin and compounds **4a–j** against HeLa, MCF-7 and A549 cancer cell lines

Table 4 Docking of thiazolo[3,2-*a*]pyrimidine molecules

Compound	Binding energy (KJ/mol)	Active site residues	No. of H-bonds
4a	− 8.4	Arg B: 241, Lys B: 321	2
4b	− 8.5	Lys A: 168	1
4c	− 8.3	Tyr B: 73, Arg B: 241	2
4d	− 8.4	–-	0
4e	− 8.7	Tyr B: 82	1
4f	− 8.0	Arg B: 242, Trp B: 62	2
4g	− 8.6	Glu A: 379	1
4h	− 8.2	Arg B: 241, Gln B: 59	2
4i	− 7.9	Arg B: 242	1
4j	− 8.1	Arg A: 324, Glu B: 379	2
Doxorubicin	− 9.8	Tyr B: 82, Gln B: 59, Arg B: 241, Tyr B: 274, Tyr B: 270, Asp B: 245, Ser B: 320, Gln B: 310	9

conventional H-bond with the amino acid Tyr B:82. Compound **4e** also interacts by making strong pi-sulfur bond with Trp B:62 and alkyl and pi-alkyl bonds with Pro B:79, Val B:57, Phe B: 77 and Met B:61, which is shown in Fig. 4, with a docking score of -8.7 compared to standard doxorubicin. Compound **4f** has formed two hydrogen bonds with nitrile and ketone groups with amino acids Arg B:242 and Trp B:62. Hydrogen of amide group in compound **4g** formed H-bond with amino acid Glu A:379 having docking score -8.6. Compound **4g** also formed strong pi-anion bond with Glu A:379, pi-sigma bond with Met A:61, pi-sulfur bond with TrpA:62 and alkyl and pi-alkyl bond with Val A:57, Phe A:77, Ile A:311Pro A:79 (Fig. 5). The most potent molecule **4e** and **4g** having two methyl groups on aromatic ring demonstrated good impact in increase of the cytotoxic activity with docking score − 8.7 and − 8.6, respectively.

Compound **4h** formed two hydrogen bonds with Gln B:59 and Arg B:241. Compound **4i** formed H-bond with Arg B:242. Compound **4j** formed H-bonding with Arg A: 324, Glu B: 379. The docking studies reveal that the thiazolopyrimidine having two CH_3 group as substitution on aryl ring in the molecule **4e** and **4g** displayed excellent inhibitory activity and interface with the target enzyme. Other functional groups, like chloro, fluoro and methoxy, interacted differently and hence showed moderate activity (Table 4).

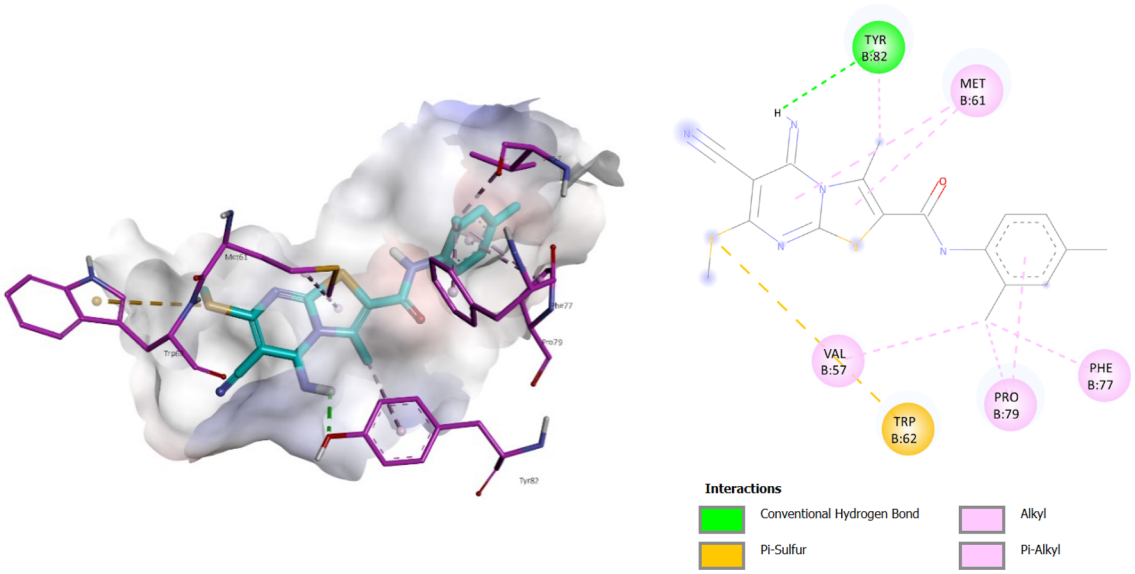


Fig. 4 Docking pose of **4e** with topoisomerase II

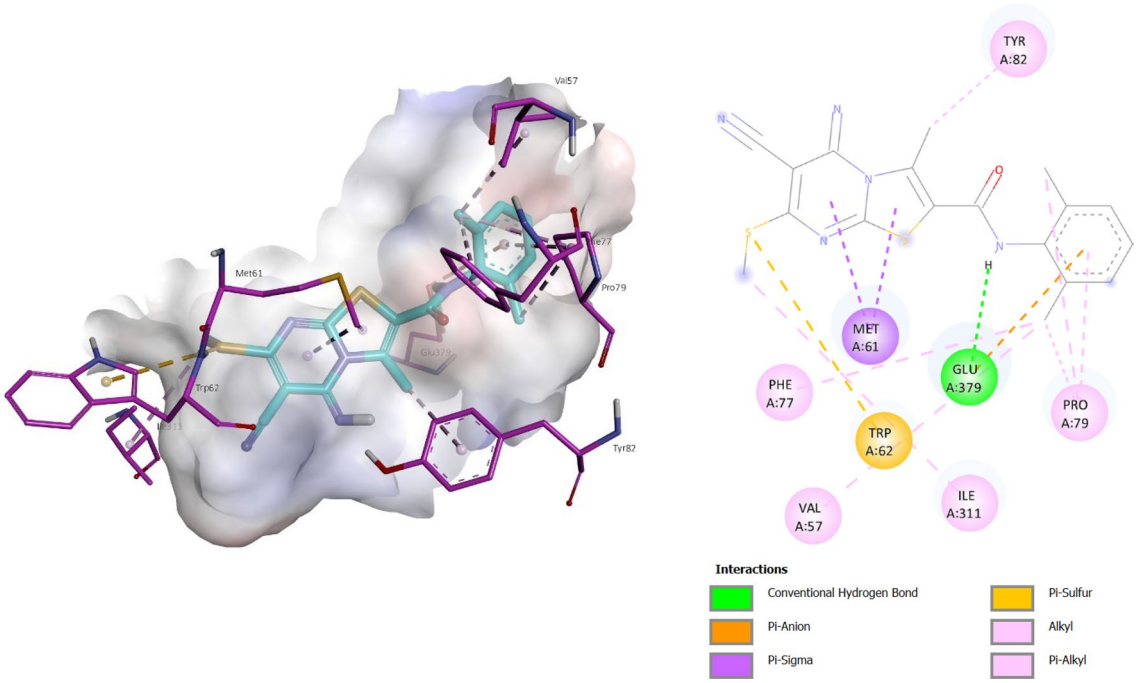


Fig. 5 Docking pose of **4g** with topoisomerase II

Experimental section

Chemistry

Melting points were determined on an electrothermal device using open capillaries and are uncorrected. Thin-layer chromatography was performed on precoated silica gel 60 F254 (Merck), and compounds were visualized with UV light at 254 and 365 nm or with iodine vapor. The IR spectra were recorded on a Shimadzu FTIR spectrometer using the ATR technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III (400 MHz) spectrometer in DMSO-d_6 or $CDCl_3$. Chemical shifts are expressed in δ ppm downfield from tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded using

a direct inlet probe on Shimadzu GCMS QP2010 Ultra mass spectrometer. All reactions were carried out under an ambient atmosphere. All reagents were purchased from Loba, Molychem, SRL and CDH and used without further purification.

General process for the synthesis of Acetoacetanilides (2a–j)

Substituted amine (10 mmol) and ethyl acetoacetate containing catalytic amount of potassium or sodium hydroxide lie (10%) in toluene were refluxed for approximately 24h. For the completion of the reaction, mass was evaporated under vacuum and the residue was crystallized from methanol or ethanol to get pure acetoacetanilides.

General process for the synthesis of thiazoles (3a–j)

To a stirred solution of compound acetoacetanilide (10 mmol) (**2a–j**) in MeOH, *N*-bromosuccinimide (15 mmol) was added and stirred at room temperature for 30 min. To this reaction, mass thiourea (20 mmol) was slowly added and refluxed for 4–5h. The reaction mixture was cooled, poured with stirring into ice water, neutralized with dilute HCl. The reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid product was filtered, washed with water and dried at room temperature to get analytically pure compound. (**3a–j**), as a light brown solid. Yield: 89% [39].

General process for the synthesis of thiazolo[3,2-*a*] pyrimidine (4a–j)

A mixture of **3a–j** (10 mmol) and 2- bis(methylthio)methylene malononitrile (10 mmol) in 10 mL of DMF and anhydrous potassium carbonate (10 mmol) was heated to reflux for 30 min. After the completion of the reaction, the reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid was filtered, washed with water and purified by recrystallization from DMF to afford crystals (**4a–j**).

6-cyano-5-imino-*N*-(4-methoxyphenyl)-3-methyl-7-(methylthio)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4a) Red crystals, yield: 88%, mp 213–215 °C with decomposition; FTIR (ATR, ν_{max}, cm^{-1}): 3281 (–NH–/=NH), 2204 (CN), 1631 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H, H-3), 9.76 (s, 1H, H24), 7.57 (d, *J* = 8.6hz, 2H, H-12, H-16), 6.96 (d, *J* = 8.7hz, 2H, H-13, H-15), 3.76 (s, 3H, H-23), 2.94 (s, 3H, H-17), 2.56 (s, 3H, H-2); MS (*m/z*): 385 (M^+); Anal. Calcd. For $C_{17}H_{15}N_5O_2S_2$: C, 52.97; H, 3.92; N, 18.17; Found: C, 52.99; H, 3.95; N, 18.19.

6-cyano-5-imino-3-methyl-7-(methylthio)-*N*-(*p*-tolyl)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4b) Yellow crystals, yield: 95%, mp 226–228 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3289 (–NH–/=NH), 2202 (CN), 1638 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H, H-3), 9.81 (s, 1H, H-23), 7.54 (d, *J* = 8.1hz, 2H, H-13, H-15), 7.19 (d, *J* = 8.1hz, 2H, H-12, H-16), 2.93 (s, 3H, H-17), 2.56 (s, 3H, H-22), 2.30 (s, 3H, H-2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.91 (C-18), 165.63 (C-5), 158.42 (C-7), 153.46 (C-20), 139.66 (C-9), 135.93 (C-14), 134.26 (C-11), 129.70 (C-13, C-15), 120.97 (C-12, C-16), 118.22 (C-21), 115.78 (C-6), 85.71 (C-19), 21.00 (C-22), 19.04 (C-17), 13.19 (C-2); MS (*m/z*): 369 (M^+); Anal. Calcd. For $C_{17}H_{15}N_5OS_2$: C, 55.27; H, 4.09; N, 18.96; Found: C, 55.25; H, 4.10; N, 18.93.

6-cyano-5-imino-3-methyl-7-(methylthio)-*N*-phenyl-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4c) Yellow crystals, yield: 91%, mp 201–203 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3284 (–NH–/=NH), 2208 (CN), 1665 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H, H-3), 7.70–7.55 (m, 3H, Ar–H), 7.39 (t, *J* = 7.8hz, 2H, Ar–H), 7.17 (t, *J* = 7.4hz, 1H, Ar–H), 2.94 (s, 3H, H-17), 2.57 (s, 3H, H-2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.96 (C-18), 165.64 (C-5), 158.65 (C-7), 153.46 (C-20), 139.79 (C-9), 138.43 (C-11), 129.33 (C-13, C-15), 125.15 (C-14), 120.96 (C-12, C-16), 118.15 (C-21), 115.76 (C-6), 85.72 (C-19), 19.03 (C-17), 13.19 (C-2); MS (*m/z*): 355 (M^+); Anal. Calcd. For $C_{16}H_{13}N_5OS_2$: C, 54.07; H, 3.69; N, 19.70; Found: C, 54.13; H, 3.72; N, 19.74.

***N*-(3-chlorophenyl)-6-cyano-5-imino-3-methyl-7-(methylthio)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4d)** Yellow crystals, yield: 93%, mp 216–218 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3202 (–NH–/=NH), 2210 (CN), 1677 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H, H-3), 9.81 (s, 1H, H-22), 7.83 (t, *J* = 2.0hz, 1H, Ar–H), 7.59 (dd, *J* = 8.1, 2.1hz, 1H,Ar–H), 7.42 (t, *J* = 8.1hz, 1H, Ar–H), 7.24 (dd, *J* = 8.1, 2.1hz, 1H, Ar–H), 2.94 (s, 3H, H-17), 2.57 (s, 3H, H-22); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.52 (C-18), 165.08 (C-5), 158.40 (C-7), 152.93 (C-20), 139.80 (C-9), 139.42 (C-11), 133.06 (C-13), 130.52 (C-15), 124.30 (C-14), 119.93 (C-12), 118.82 (C-16), 117.28 (C-21), 115.18 (C-6), 85.27 (C-19), 18.56 (C-17), 12.72 (C-2); MS (*m/z*): 389 (M^+); Anal. Calcd. For $C_{16}H_{12}ClN_5OS_2$: C, 49.29; H, 3.10; N, 17.96; Found: C, 49.32; H, 3.14; N, 17.97.

6-cyano-*N*-(2,4-dimethylphenyl)-5-imino-3-methyl-7-(methylthio)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4e) Yellow crystals, yield: 90%, mp 242–244 °C; FTIR (ATR, ν_{max}, cm^{-1}): 2210 (CN), 1679 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H, H-3), 9.41 (s, 1H, H-22), 7.23 (d, *J* = 8.0hz, 2H, Ar–H), 7.11 (s, 1H, Ar–H), 7.05 (d, *J* = 7.9hz, 2H, Ar–H), 3.00 (s, 3H, H-26),

2.56 (s, 3H, H-24), 2.29 (s, 3H, H-2), 2.20 (s, 3H, H-23); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.38 (C-18), 165.07 (C-5), 158.42 (C-7), 152.99 (C-20), 139.66 (C-9), 135.85 (C-14), 133.17 (C-16), 132.65 (C-11), 131.02 (C-15), 126.70 (C-13), 126.09 (C-27), 117.28 (C-12), 115.29 (C-6), 85.23 (C-19), 20.52 (C-24), 18.51 (C-23), 17.88 (C-26), 12.68 (C-2); MS (*m/z*): 383 (M^+); Anal. Calcd. For $C_{18}H_{17}N_5OS_2$: C, 56.38; H, 4.47; N, 18.26; Found: C, 56.40; H, 4.52; N, 18.29.

***N*-(4-chlorophenyl)-6-cyano-5-imino-3-methyl-7-(methylthio)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4f)** Yellow crystals, yield: 89%, mp 230–232 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3268 (–NH–/=NH), 2202 (CN), 1639 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H, H-3), 9.96 (s, 1H, H-20), 7.69 (d, *J* = 8.9hz, 2H, Ar–H), 7.45 (d, *J* = 8.9hz, 2H,Ar–H), 2.93 (s, 3H, H-23), 2.56 (s, 3H, H-2); MS (*m/z*): 389 (M^+); Anal. Calcd. For $C_{16}H_{12}ClN_5OS_2$: C, 49.29; H, 3.10; N, 17.96; Found: C, 49.24; H, 3.06; N, 17.92.

6-cyano-*N*-(2,6-dimethylphenyl)-5-imino-3-methyl-7-(methylthio)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4g) Yellow crystals, yield: 92%, mp 238–240 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3218 (–NH-/=NH), 2201 (CN), 1630 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H, H-3), 9.31 (s, 1H, H-20), 7.16 (s, 3H, Ar–H), 3.04 (s, 3H, H-24), 2.56 (s, 3H, H-2), 2.21 (s, 3H, H-21, H-22); MS (*m/z*): 383 (M^+); Anal. Calcd. For $C_{18}H_{17}N_5OS_2$: C, 56.38; H, 4.47; N, 18.26; Found: C, 56.36; H, 4.43; N, 18.24.

6-cyano-*N*-(4-fluorophenyl)-5-imino-3-methyl-7-(methylthio)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4h) Yellow crystals, yield: 90%, mp 237–239 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3310 (–NH-/=NH), 2208 (CN), 1620 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H, H-3), 9.93 (s, 1H, H-20), 7.68 (dd, *J* = 9.1, 5.0hz, 2H, Ar–H), 7.24 (t, *J* = 8.9hz, 2H, Ar–H), 2.94 (s, 3H, H-23), 2.56 (s, 3H, H-2); MS (*m/z*): 373 (M^+); Anal. Calcd. For $C_{16}H_{12}FN_5OS_2$: C, 51.46; H, 3.24; N, 18.75; Found: C, 51.50; H, 3.22; N, 18.76.

***N*-(4-bromophenyl)-6-cyano-5-imino-3-methyl-7-(methylthio)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4i)** Yellow crystals, yield: 95%, mp 248–250 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3309 (–NH–/=NH), 2207 (CN), 1680 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H, H-3), 10.00 (s, 1H, H-20), 7.64 (d, *J* = 8.9hz, 2H, Ar–H), 7.58 (d, *J* = 8.9hz, 2H, Ar–H), 2.93 (s, 3H, H-23), 2.56 (s, 3H, H-2); MS (*m/z*): 434 (M^+); Anal. Calcd. For $C_{16}H_{12}BrN_5OS_2$: C, 44.25; H, 2.78; N, 16.12; Found: C, 44.27; H, 2.79; N, 16.15.

6-cyano-5-imino-*N*-(2-methoxyphenyl)-3-methyl-7-(methylthio)-5*H*-thiazolo[3,2-*a*]pyrimidine-2-carboxamide (4J) Yellow Crystals, Yield: 94%, mp 212–214 °C; FTIR (ATR, ν_{max}, cm^{-1}): 3291 (–NH–/=NH), 2201 (CN), 1619 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H, H-3), 9.03 (s, 1H, H-20), 7.68 (d, *J* = 7.8hz, 1H, Ar–H), 7.24 (td, *J* = 7.8, 1.7hz, 1H, Ar–H), 7.12 (dd, *J* = 8.3, 1.4hz, 1H, Ar–H), 6.99 (td, *J* = 7.7, 1.4hz, 1H, Ar–H), 3.84 (s, 3H, H-22), 3.00 (s, 3H, H-24), 2.56 (s, 3H, H-2); MS (*m/z*): 385 (M^+); Anal. Calcd. For $C_{17}H_{15}N_5O2S_2$: C, 52.97; H, 3.92; N, 18.17; Found: C, 52.95; H, 3.89; N, 18.14.

Prediction of the physicochemical properties

The computational data are displayed in Table 5, which was carried out using SwissADME. On the description of these data, Lipinski's rule of five is not violated [40]. It is assumed that the prepared molecule scan has a decent pharmacokinetic outline. Therefore, the molecules could be useful for drug-likeness profile.

Experimental protocol of molecular docking study

The ChemSketch 2021.2.0 software was used for the generation of ligand structures. Furthermore, the energy minimization of every molecule was performed using the Dundee PRODRG2 server, and AutoDock Vina 1.1.2 was used to for the docking studies [41]. Topoisomerase II alpha was downloaded from PDB database (1ZXM). Heteroatoms were excluded to make the structure receptor free from all ligands prior to docking. The protein preparation was done by addition of Kollaman charge, solvation parameters and polar hydrogens. The grid box size was set to 40, 40 and 40 Å for x, y and z correspondingly. The grid center was set to 49.588, 3.725 and 22.091 for x, y and z individually. The spacing among grid points was 0.375 angstroms, and the exhaustiveness was equal to 40. Powerful molecular graphics viewer, Discovery Studio Visualizer v21.0, was used to figure out the most probable binding mode [42].

Cytotoxic evaluation

Materials and methods

Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM) and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were purchased from Sigma-Aldrich Chemicals. Ninety-six-well cell culture plates were purchased from Thermo Fisher Scientific. Topoisomerase II, human lung adenocarcinoma cell line (A549), human breast carcinoma cell line (MCF-7) and human cervical cancer cell line (HeLa) were obtained from the American Type Culture Collection (ATCC), India.

Table 5 Physicochemical, pharmacokinetic and medicinal chemistry properties of the synthesized molecules 4a–j

Physicochemical properties						Pharmacokinetics		Medicinal chemistry	
Compound	HBA	HBD	TSPA	Log $P_{o/w}$	Log S	GIA	Log K_p	RoF (V)	SA
4a	5	2	156.81	2.50	− 5.24	Low	− 7.01	Yes (0)	3.24
4b	4	2	147.58	2.81	− 5.45	Low	− 6.64	Yes (0)	3.2
4c	4	2	147.58	2.45	− 5.07	Low	− 6.81	Yes (0)	3.14
4d	4	2	147.58	3.03	− 5.72	Low	− 6.58	Yes (0)	3.14
4e	4	2	147.58	3.16	− 5.83	Low	− 6.47	Yes (0)	3.33
4f	4	2	147.58	3.04	− 5.72	Low	− 6.58	Yes (0)	3.13
4g	4	2	147.58	3.12	− 5.83	Low	− 6.47	Yes (0)	3.33
4h	5	2	147.58	2.78	− 5.17	Low	− 6.85	Yes (0)	3.12
4i	4	2	147.58	3.12	− 5.78	Low	− 6.81	Yes (0)	3.16
4j	5	2	156.81	2.51	− 5.24	Low	− 7.01	Yes (0)	3.25
Doxorubicin	12	6	543.52	0.5	− 5.2	Low	− 8.71	No (3)	5.81

HBA H-bond acceptor, *HBD* H-bond donor, *TPSA* topologic polar surface area, *Log* $P_{o/w}$ lipophilicity, *Log S* water solubility, *GIA* gastrointestinal absorption, *Log* K_p skin permeation, *RoF (V)* Lipinski's rule of five, *SA* synthetic accessibility

Cell culture

Cell lines were cultured in DMEM medium containing 10% fetal bovine serum, 10 ml/L antibiotic–antimycotic solution (10,000 Units/ml penicillin, 10 mg/L streptomycin and 25 µg/mL amphotericin B), and this culture was kept in CO_2 incubator with 90% humidified atmosphere and 5% CO_2 at 37 °C.

Preparation of samples for MTT assay

DMSO (dimethyl sulfoxide) was used to dissolve test compounds **4a–j** with a stock concentration of 10 µM. Subsequently, with sterile PBS (1X) dilutions were made to achieve. The dilutions were made with sterile PBS (1X) to get chosen concentrations. This was followed by filtration with a 0.22-µm sterile filter and subjected to exposure to UV light for 20 min. Then, each sample was added to a 96-well microtiter plate containing cells.

MTT assay

MTT assay is used to determine the cell viability, thereby analyzing the cytotoxicity of synthesized molecules. Here, the protocol followed is referenced by Sekhar [17]. The exponentially dividing cell concentration of 5×10^3 was seeded into the microtiter plate (counted by trypan blue exclusion dye method). Further, cells were grown till 60–70% confluence after which compounds **4a–j** with the final concentration ranging from 0.1, 1, 5 and 10 µM were added to the culture medium, and as a positive control, doxorubicin was utilized and DMSO was added to negative control and incubation for 24 and 48h at 37 °C with 5% CO_2 and 90% humidified atmosphere in a CO_2 incubator was performed. Soon after, the media of the wells were replaced with 90 µL of fresh serum-free media and 10 µL of MTT (5 mg/mL of PBS) and again incubated at 37 °C for 2h, whereas the above media was disposed, and the plate was dried for 30 min. Then into each well, 100µL of DMSO was added at 37 °C and continued for 5 min. The purple formazan crystals were dissolved and were instantly measured on SpectraMax Plus 384 UV–visible plate reader at 570 nm. The absorbance values relative to the control were used to determine IC_{50} values (Table 3).

Conclusion

In conclusion, we have synthesized few novel hybrid molecules bearing thiazole and pyrimidine moieties. The synthesized compounds have been characterized by various analytical techniques such as FTIR MS, ^{1}H NMR and ^{13}C NMR. Anticancer activity of these novel synthesized molecules was evaluated on the human lung adenocarcinoma cell line (A549), human cervical cancer cell line (HeLa) and human breast carcinoma cell line (MCF-7) using the MTT assay. The present synthetic route is simple, novel, reasonable experimental process, quick reaction time and having excellent yield. In addition, few of the prepared molecules exhibited good-to-moderate anticancer activity. Among all the tested compounds, compounds **4e** and **4g** substituted with the CH_3 group demonstrated the maximum cytotoxic activity. Docking studies of prepared molecules **4a–j** showed the compounds **4b**, **4e** and **4g** displayed the top docking value and indicated that **4e** and **4g** bind well with the topoisomerase II. Research outcome of this study

can be helpful for preparing new anticancer molecules that is effective in cancer therapy.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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